Pestic. Sci. 1996, **48**, 325–332

Stability and Biological Activity of Cyfluthrin Isomers†

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(Received 27 February 1996; revised version received 10 July 1996; accepted 9 August 1996)

Abstract: A GC-MS method capable of completely separating the four pairs of diastereoisomers of cyfluthrin is presented and the method used to show that isomerisation of the cyfluthrin enantiomers occurs in methanol. This methanol-induced isomerisation could also be demonstrated by bioassays using water fleas. The biological activities of the various cyfluthrin isomers contained in the commercial products cyfluthrin and beta-cyfluthrin were assayed using several strains of lepidopteran larvae including *Plutella xylostella*, *Heliothis virescens* and *Spodoptera frugiperda*. With the susceptible strains, the efficiencies of the isomers mixtures of cyfluthrin and beta-cyfluthrin were shown to obey the rules of additivity. However, in tests with a resistant strain of *P. xylostella* originating from Thailand, the 'inactive' isomer III acted synergistically with the active isomer IV. Resistance factors in strains of *H. virescens* and *P. xylostella* were found to be higher with *cis* than with *trans* isomers. This probably contributes to the superior action of cyfluthrin and beta-cyfluthrin against various pests of agricultural importance since the commercial products contain a high content of *trans* isomers ('high *trans* pyrethroids').

Key words: Cyfluthrin, beta-cyfluthrin, isomers, isomerisation, diastereoisomers, *Plutella xylostella*, *Heliothis virescens*, *Spodoptera frugiperda*, *Daphnia magna*

1 INTRODUCTION

The chemical structure of the cyfluthrin molecule is shown in Plate 1 from which it can be seen that the asymmetric carbon atoms at positions 1, 3 and alpha (marked yellow) lead to eight enantiomeric forms of the molecule. These enantiomers can be grouped into four pairs. With respect to the plane of the cyclopropane ring, two pairs of enantiomers have the *cis* and the other two have the *trans* conformation. Cyfluthrin, the synthesis of which leads to the formation of a mixture of all possible enantiomers, is sold as an insecticide under the trade name 'Baythroid' (Plate 1) and was first described by Hammann and Fuchs.[1] Plate 1 shows the relative percentage of the enantiomers in a typical batch of this product. In this paper, the diastereomeric pairs of cyfluthrin will be referred to as isomers **I** to **IV**. The assignment of numbers to the isomers is based on the relative retention volumes obtained in HPLC experiments. Usually, it is assumed that only two of these enantiomers (marked red) contribute directly to the biological performance[2] against most species of pest organisms. Therefore, an optimised insecticide, beta-cyfluthrin ('Bulldock'), in which the proportion of the active enantiomers has been enriched, was developed. In this paper, methods for the chromatographic separation of the isomers, tests for their individual biological activities and an evaluation of the effect of diastereoisomeric composition on biological activity are discussed. The test organisms used were larvae of the Lepidopterae *Plutella xylostella* (L.), *Heliothis virescens* (F.) and *Spodoptera frugiperda* (Smith).

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† One of a collection of papers on various aspects of pest control research contributed by staff of Bayer AG and collated by Dr M. Londershausen and Dr A. Turberg.

2 EXPERIMENTAL

2.1 Chemicals

The cyfluthrin used for the experiments with *P. xylostella*, contained 26·3, 22·3, 26·2 and 25·2% (w/w) of

Pestic. Sci. 0031-613X/96/$09.00

isomers **I** to **IV**, respectively but those with *H. virescens* and *S. frugiperda* involved a batch of cyfluthrin containing 25·1, 19·0, 33·5 and 22·4% (w/w), respectively. The latter batch was also used for the analytical separation. The composition of beta-cyfluthrin was 31·7% (**II**) and 68·3% (**IV**). The purity of the isolated isomers was > 98% (HPLC). The metabolites methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and 3-phenoxy-4-fluorobenzaldehyde were synthesised by known methods and their purity was >95%. All solvents used were of analytical grade.

2.2 Gas chromatography

The mixture of the cyfluthrin isomers (37·5 ng) was separated on a HP-1 capillary column (0·2 mm ID; 0·33 μm film thickness; 12·5 m long). The gas chromatograph (HP 5890) was on-line coupled to a mass-selective detector (HP 5970) and an automatic sampling device (HP 7673A). A temperature gradient program with a helium flow of 0·9 ml min^{-1} resulted in a clear separation of the four pairs of enantiomers (see Fig. 2). The amplifier unit of the detector was set to a range from 50 to 550 amu and the scan rate was 1·7 per second.

2.3 Isomerisation

The experiments used to investigate isomerisation were performed in (A) hexane, (B) methanol and (C) methanol + water (9 + 1, by volume) at an isomer concentration of 1·5 g $litre^{-1}$ (see Fig. 3). The solutions were incubated for 48 h at 25°C prior to GC analysis as described above. The metabolites methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (permethrin acid methyl ester) and 3-phenoxy-4-fluorobenzaldehyde, and isomer **II** were identified by co-chromatography with reference substances and by comparison of their mass spectra.

2.4 Bioassays with waterfleas

Waterfleas (*Daphnia magna*, Straus) were reared under laboratory conditions using algae (*Scenedesmus subspicatus*, Chodat) as food source in a standardised isotonic medium.

Bioassays were performed in 96-well trays. One healthy adult waterflea in its isotonic medium was added to yield a total volume of 300 μl in each well. Solutions of the isomers in methanol (1 mg $litre^{-1}$; 5 μl), either prepared immediately before use or after incubation at 25°C for 96 h, were added to the isotonic medium in each well of the 96-well tray giving a final concentration of 17 μg $litre^{-1}$. During exposure to the isomers, the waterfleas were inspected for the onset of lasting irregular spasmodic cramps after 15, 30, 60, 120, 240, 480 min and again after 24 h. All experiments were performed in duplicate and controls showed that neither mortality nor symptoms were observed if the solvent methanol was tested under the same conditions.

2.5 Insects

2.5.1 Lepidopteran larvae

A susceptible strain of *P. xylostella* (SS) originating from Northern Germany, which has been reared in the laboratory since 1955, was used in the tests. A resistant strain (LTD) reared in the laboratory, was obtained from Thailand in 1979. Its resistance was maintained by regular exposure to methomyl, methamidophos and DDT using a rotational scheme. A resistant field strain, Bang I, was collected from the field at a location near Bangbuathong (Thailand) in 1986. This strain was reared without any further insecticide treatment. Bang II was obtained from the same location in 1987, and reared under the same conditions as Bang I.

The strain of *S. frugiperda* used was a susceptible one (SS) originating from Jordan; it has been reared in the laboratory since 1967.

Both strains of *H. virescens* (SS and PEG) were obtained from Dr McCaffery, University of Reading, UK and originated in the USA.

2.5.2. Bioassays with lepidopteran larvae

Freshly prepared solutions of the cyfluthrins or their isomers in acetone were applied topically (0·5 μl) to 4th-instar larvae (3rd-instar larvae for *Spodoptera*). Their effect in terms of inability to feed on leaves was rated after 5–24 h exposure to the test material at 20°C. Leaf discs of appropriate size (maize for *Spodoptera*, cabbage for *Plutella* and cotton for *Heliothis*) were mounted on a thin agar (1%) layer in 24-well trays. Ten larvae (two in each well in the case of *Plutella* and *Heliothis*, and one larva in the case of *Spodoptera*) were treated per concentration, and four to five concentrations were assayed per experiment. Each experiment also included solvent controls. Two to three independent experiments were used as a basis for the calculation of an LD_{50} value.

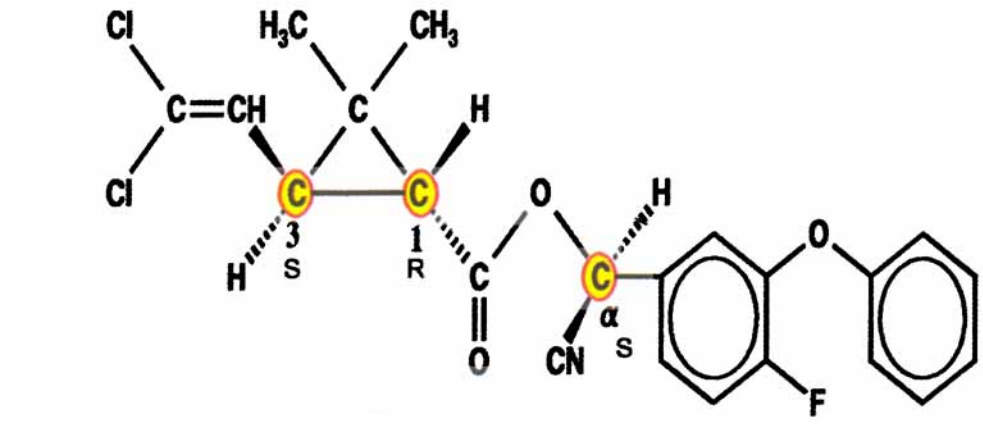
Probit analyses[3] were performed using a computer program compiled for a HP 86 laptop computer (Leicht, 1982, unpublished). Fiducial limits (95%) are given.

2.6 Joint action in mixtures

The calculation of the theoretical activity of cyfluthrin and beta-cyfluthrin was performed using the formula for joint action described by Carpenter *et al.*[4]

$$LD_{50mix} = \left[\frac{P_a}{LD_{50a}} + \frac{P_b}{LD_{50b}} + \cdots \frac{P_n}{LD_{50n}}\right]^{-1} \quad (1)$$

where P*a*, *b*, ... *n* represent the fractions of the individual components (isomers) in the mixture.



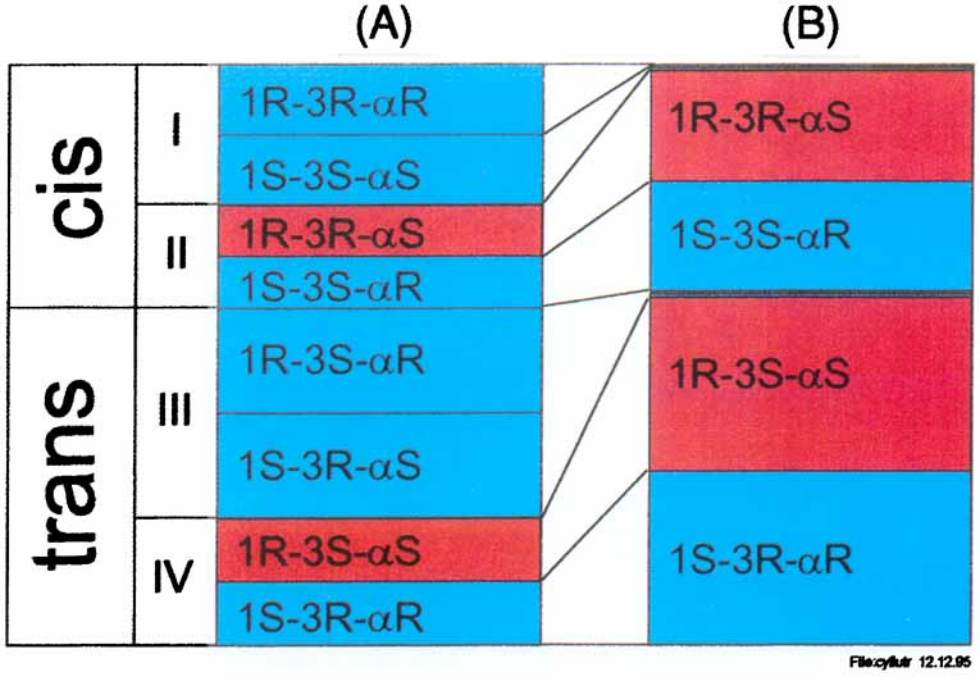


Plate 1. Stereochemistry of cyfluthrins. The sizes of the shaded areas indicate the percentage composition of each isomer in a typical batch of (**A**) technical cyfluthrin, (**B**) technical beta-cyfluthrin. Blue and red represent the biologically inactive and active isomers respectively.

3 RESULTS

3.1 Separation of cyfluthrin isomers

The different physical properties of the diastereomeric pairs of cyfluthrin allowed their analytical separation using various techniques. The most powerful tool routinely used for separation was a gas chromatograph equipped with a capillary column and a mass selective detector. Figure 1 shows a typical chromatographic separation of the four diastereomeric pairs of cyfluthrin. The inactive isomers, **I** and **III**, eluted ahead of the biologically active isomers, **II** and **IV**. The larger quantities of the *trans* isomers, the inactive isomer **III** and active isomer **IV**, relative to the *cis* isomers, the inactive isomer **I** and the active isomer **II**, was also reflected by their peak heights. It was also possible to resolve this isomeric mixture by high performance liquid chromatography (HPLC) on reversed-phase silica gel columns (chromatogram not shown). With this technique, the *cis* isomers **I** and **II** eluted before the *trans* isomers (**III** and **IV**). Conventional thin-layer chromatography with a single solvent mixture did not yield a complete separation of the four diastereomeric pairs. However, the *cis* and the *trans* isomer mixtures could be separated by TLC using a multisolvent gradient technique (TLC-AMD)[5] on silica plates (chromatogram not shown). In this system, the individual *cis* isomers did not separate and had a higher R_f value than the *trans* isomers, which were also not separated into individual isomers.

3.2 Isomerisation

In aprotic solvents such as hexane, acetonitrile and dichloromethane at room temperature and in the absence of light, the cyfluthrin isomers were stable and isomerisation was not observed. As an example, isomer **I** remained unchanged after storage in hexane at 25°C for 48 h (Fig. 2(A)). However, if isomer **I** was incubated in methanol or methanol + water at room temperature and in the dark a fairly rapid isomerisation was observed; as shown in Figs 2(B), (C) an additional peak, with higher retention time, appeared. This was identified as isomer **II**. In addition to the isomerisation, hydrolysis apparently also took place. When isomer **I** was incubated in methanol (Fig. 2(B)) there was a time-dependent increase of polar peaks (retention times 3·8 and 4·7 min) while in methanol + water (9 + 1 by volume), hydrolysis proceeded at a faster rate, as is shown in Fig. 2(C). The hydrolysis products were identified as 3-phenoxy-4-fluorobenzaldehyde and methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate.

The temperature dependence of this isomerisation reaction was also investigated using a constant incubation time of 20 h (data not shown). At 2°C, isomer **I** was stable in both hexane and methanol; however, in methanol at 10°C, traces of isomer **II** could be detected. At 22°C, isomers **I** and **II** were present in equal amounts and at 40°C, the hydrolysis products as above were the dominant peaks in the chromatogram. In hexane, isomer **I** remained stable at all temperatures.

3.3 Influence of isomerisation on the activity of cyfluthrin isomers against waterfleas

Changes in toxicity of cyfluthrin isomers before and after incubation in methanol were assayed using a test system containing waterfleas (*D. magna*). Table 1 shows the results of these experiments. Without incubation in methanol or with very short incubation periods, an

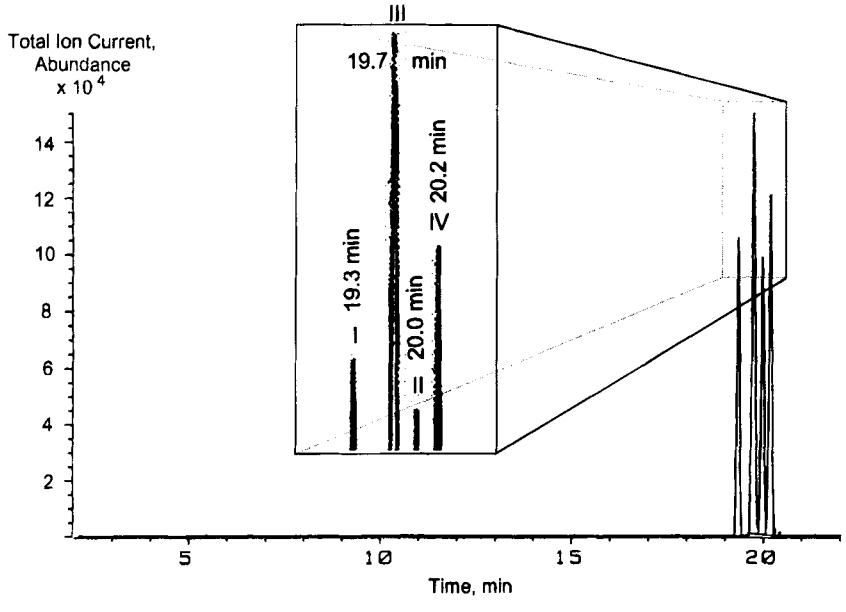


Fig. 1. Gas chromatographic separation of cyfluthrin. The Roman numbers in the enlargement refer to the diastereomeric pairs of cyfluthrin (see Plate 1).

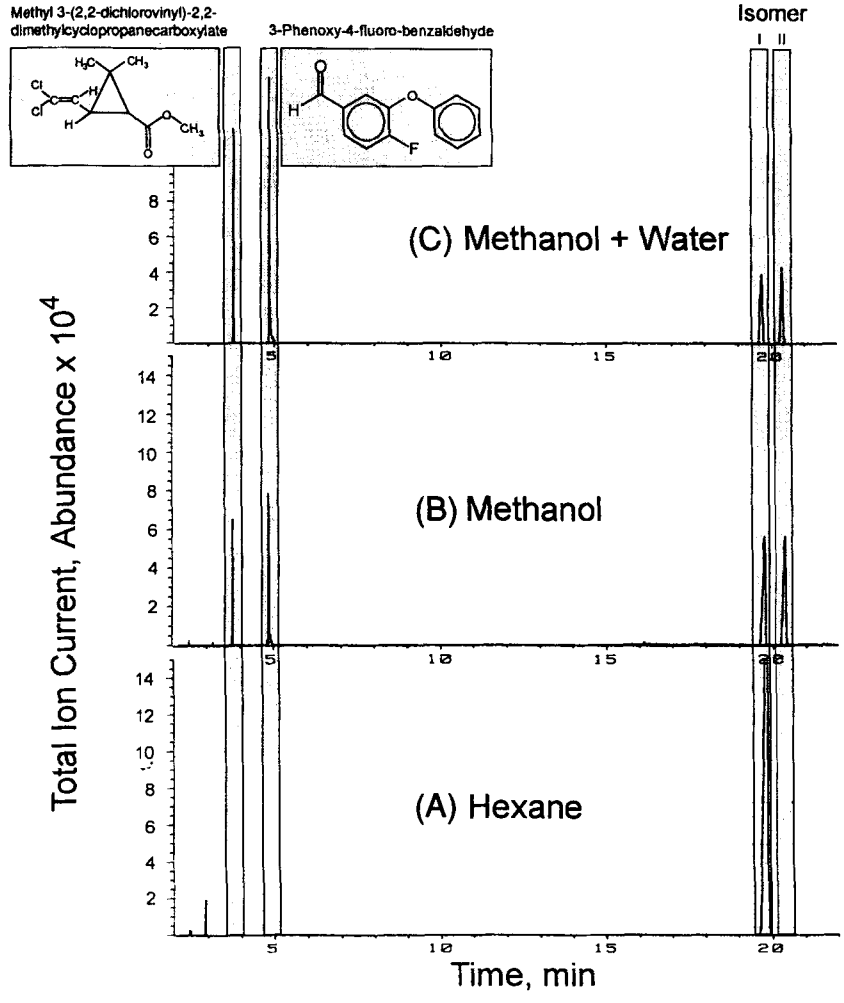


Fig. 2. Isomerisation and degradation of cyfluthrin isomer I after incubation in methanol and methanol + water. The isomer was dissolved in (A) hexane, (B) methanol or (C) methanol + water (9 + 1, by volume) and the solution was incubated for 48 h at 25°C prior to GC analysis.

activity pattern was observed which was similar to that in experiments where aprotic solvents were used (e.g. topical application with lepidopteran larvae). Activity was first observed with the isomer **II** and subsequently also with isomer **IV** but isomers **I** and **III** assayed at the same concentration showed no activity within 24 h. However, after each of the four isomers was incubated in methanol at room temperature for 96 h, the differences between the biological activities of the resulting samples were much smaller. For example, while incubation of the active isomers **II** and **IV** in methanol retarded the onset of activity, the converse was true with isomers **I** and **III**.

TABLE 1
Changes in Biological Activity against Waterfleas of Cyfluthrin Isomers after Incubation in Methanol[a]

	Time taken for symptoms to appear (h)	
Isomer	*Non-incubated*	*Incubated*
I	>24	2
II	0·25	4
III	>24	4
IV	2	24

[a] Isomers incubated in methanol for 96 h at 25°C prior to the assay.

3.4 Activities of the isomers against lepidopteran larvae

A survey of the biological activity of the cyfluthrins and their isomers on three species of lepidopteran larvae is given in Table 2. In all seven strains investigated, it became evident that the isomers **I** and **III** were much less biologically active than the isomers **II** and **IV**.

In this context, no general statement can be made about the toxicity of the various isomeric configurations. In all strains of *P. xylostella*, the *trans* isomer was more active than the *cis* isomer. In contrast to this, with larvae of *S. frugiperda* and *H. virescens*, the *cis* isomer was more active. If a comparison was made between susceptible and resistant strains of the *Plutella* and *Heliothis* species, the *trans* isomer became more effective than the *cis* isomer on a relative basis. The

TABLE 2
Biological Activity of the Isolated Isomers, Cyfluthrin and Beta-Cyfluthrin on Various Strains of Lepidopteran Larvae. Fiducial Limits (95%) are given in Brackets

Target insect and strain	LD_{50} *(ng per larva)*					
	Isolated isomers				*Cyfluthrin*	*beta-Cyfluthrin*
	Cis *isomers*		Trans *isomers*			
	I	**II**	**III**	**IV**	**I + II + III + IV**	**II + IV**
Plutella xylostella (Diamondback Moth)						
SS[a]	68 (49–93)	1·7 (1·3–2·1)	33 (26–42)	0·97 (0·67–1·5)	2·6 (1·5–4·3)	0·98 (0·65–1·4)
LTD	7800	68 (42–110)	3500 (1300–9900)	33 (21–52)	67 (43–100)	32 (14–68)
Bang I	17 000 (7000–50 000)	190 (66–520)	2700 (1400–5300)	85 (20–230)	160 (110–200)	120 (48–320)
Bang II	3600 (1500–8900)	49 (18–150)	470 (87–1400)	20 (6–50)	66 (34–130)	39 (23–68)
Spodoptera frugiperda (Fall Armyworm)						
SS[a]	18 (11–39)	0·71 (0·36–1·3)	170 (100–280)	42 (24–91)	2·7 (1·5–4·7)	2·8 (1·5–5·2)
Heliothis virescens (Tobacco Budworm)						
SS[a]	19 (13–28)	0·44 (0·19–1·1)	36 (25–88)	6·1 (2·6–21)	2·3 (0·77–6·9)	nt[b]
PEG	30000 (extrapolated)	18 (9·7–32)	90000 (extrapolated)	23 (11–47)	71 (23–450)	nt

[a] Susceptible strain.
[b] nt: not tested.

latter tendency was quite obvious if the resistance factors for the *cis* and *trans* isomers were compared (Table 3). In all cases, the resistance factors of the *trans* isomer were smaller than those of the *cis* isomer.

3.5 Synergistic action of inactive enantiomers

If the activities of the individual isomers are known, the expected activity for isomer mixtures such as cyfluthrin and beta-cyfluthrin can be calculated using eqn (1). If the individual isomers do indeed contribute additively to toxicity, the calculated and experimentally determined efficacies of isomer mixtures such as cyfluthrin should coincide. Such data are given in Table 4 for cyfluthrin and beta-cyfluthrin with susceptible and resistant strains of *P. xylostella* and *H. virescens*. In the case of the susceptible strains of *P. xylostella* and *S. frugiperda*, calculated and experimental values were very similar. For example, with *P. xylostella*, the experimentally determined LD_{50} of 2·6 ng per larva was very close to the calculated value of 2·5 ng per larva (Table 4) for cyfluthrin. This close agreement strongly suggests that the isomers act additively. The same comparison can be made with beta-cyfluthrin. Here, there was also a very close agreement between the experimentally determined LD_{50} (0·98 ng per larva) and the calculated LD_{50} (1·1 ng per larva).

In the case of certain resistant strains of insects, the simple rules of additivity were not always obeyed. For the highly resistant strain of *P. xylostella* (Bang I), the LD_{50} of 160 ng per larva found experimentally indicated activities higher than that suggested by the calculated LD_{50} value of 240 ng per larva.

To assess the possible role of 'inactive' isomers on the

TABLE 3
Comparison of Resistance Factors for the *cis* and *trans* Isomers in Various Species

Species	*Factor of resistance*		*Ratio*
	Isomer II cis	*Isomer IV* trans	*II/IV*
P. xylostella (LTD)	40	34	1·2
P. xylostella (Bang I)	112	88	1·3
P. xylostella (Bang II)	29	21	1·4
H. virescens	41	3·8	11

TABLE 4
Comparison of Experimentally Obtained and Calculated Efficiencies of Cyfluthrin and beta-Cyfluthrin. Experimental Data taken from Table 2

	Cyfluthrin LD_{50} [*ng per larva*]		*beta-Cyfluthrin* LD_{50} [*ng per larva*]		*Activity ratio*[a]	
	Experimental	*Calculated*[b]	*Experimental*	*Calculated*[b]	*Experimental*	*Calculated*
P. xylostella						
SS[c]	2·6	2·5	0·98	1·1	2·7	2·3
LTD	67	91	32	39	2·1	2·3
Bang I	160	240	120	100	1·3	2·4
Bang II	66	56	39	25	1·7	2·2
S. frugiperda						
SS[c]	2·7	3·5	2·7	2·2	1·0	1·6
H. virescens						
SS[c]	2·3	2·0				
PEG	71	49				

[a] Ratio LD_{50} cyfluthrin/LD_{50} beta-cyfluthrin.
[b] Calculated according to eqn (1).
[c] Susceptible strain.

efficacy of active cyfluthrin isomers, dose–response experiments with isomer **IV** were performed in the presence and absence of the isomers **I** and **III**. In these tests, the same resistant strain (Bang I) was used. In the presence of isomer **III** (300 ng per larva) the LD_{50} of isomer **IV** was lowered by a factor of five. If, however, isomer **III** was replaced by isomer **I**, no significant change of the activity of isomer **IV** was observed.

4 DISCUSSION

4.1 Analytical aspects

HPLC and capillary gas chromatography were equally capable of separating the cyfluthrin isomers. The separation of the cyfluthrin isomers by HPLC has been reported in the literature by Slahck,[6] Abidi[7] and Chapman.[8] A gas chromatographic method was published by Dicke *et al.*[9] If the isomers are to be analysed in biological matrices without excessive clean-up, the GC-MS technique is superior to all other methods. For separation of all eight cyfluthrin isomers, chiral phases must be used. For several pyrethroids (including cyfluthrin), Lisseter and Hambling[10] showed that HPLC on a chiral column is capable of resolving all isomers.

4.2 Isomerisation

From the results obtained thus far, it is not entirely clear if the activity observed in experiments with the 'inactive' isomers was due to small traces of active isomers, to an inherent activity of the isomers themselves or to isomerisation.

However, it could be shown that cyfluthrins in protic organic solvents, such as methanol, are susceptible to isomerisation, both analytically (Fig. 1) and by bioassay (Table 1). In the latter case, and depending on the isomer tested, activation and inactivation was a consequence of an isomerisation which was catalysed by methanol. Under the conditions chosen, the inactive isomers **I** and **III** became activated. This is in agreement with the hypothesis that isomers **I** and **III** can be isomerised to isomers **II** and **IV**, respectively. The observation that biologically active isomers **II** and **IV** can be transformed to the inactive isomers **I** and **III** is further evidence for the isomerisation process.

Although the effect of isomerisation in protic solvents can be taken as an established fact, it is difficult to design experiments which would show whether or not the biological effects of the isomers **I** and **III** are also due to isomerisation within living organisms.

In at least one case, analytical evidence could be given that isomerisation can also occur under field conditions (Lafuerza and Leicht, unpublished). For example, in Southern France, cotton plants, grown in an experimental field plot near Montpellier, were sprayed with EC and SC formulations of beta-cyfluthrin. At various time intervals, plants were removed and analysed for their content of cyfluthrin isomers. Surprisingly, in the case where the leaves were sprayed with the EC formulation, a time-dependent increase of isomers **I** and **III** was observed; these particular isomers are usually not present in formulations of

beta-cyfluthrin. Their occurrence is most likely due to an isomerisation similar to that observed in methanol. No traces of isomers **I** and **III** were found when cotton leaves were sprayed with an SC formulation. Apparently, the cyfluthrin molecules within the micro-crystals of the SC-formulation are less exposed to protic and isomerisation-promoting environments.

4.3 Functions of inactive isomers

As shown in Table 2, isomers **II** and **IV** can be regarded as the active ingredients of cyfluthrin. In screening experiments, this observation was made for most of the many insect species tested (Bayer, unpublished). It is also in agreement with a study on the cotton stainer, *Dysdercus albofasciatus* (Berg), published by Stadler and Schang.[11]

Using eqn (1) and the activities of the isomers given in Table 1, the importance of the 'inactive' isomers (**I** and **III**) for the performance of cyfluthrin could be evaluated. Assuming that these isomers were totally inactive, this would lead to an increase in the LD_{50} value from 2·5 to 2·6 ng per larva. Thus, the contribution of the isomers **I** and **III** to the performance of cyfluthrin is negligible, and these isomers are regarded as 'inactive'. In spite of this, their biological activity can be characterised by LD_{50} and LD_{95} values.

Referring back to Plate 1, it can be seen that the inactive isomers **I** and **III** are major components of cyfluthrin. As discussed above, these isomers do not contribute to the field performance of the product against the susceptible strain, *P. xylostella*, and a number of other species. Historically, after detection of the differences in the activities of the four isomers, this was the impetus to develop an improved cyfluthrin product, beta-cyfluthrin. This product was considered a 'better' cyfluthrin, since the active isomers were enriched, and the inactive isomers, which were often regarded as 'isomeric ballast and environmental pollutants' (Ariens *et al.*[12]) were eliminated. This rather simplistic picture was first challenged when beta-cyfluthrin did not show the expected increase of efficiency under field conditions. Detailed investigations showed that the simple rules of additivity did not apply when dealing with the control of some resistant insect strains. Investigations with the resistant strain of diamondback moth, (Bang I), showed that cyfluthrin was significantly more active than could be expected from the additive activities of its constituent isomers. Further, it could be shown that isomer **III** synergised the activity of isomer **IV**. This is consistent with the observation that synergism was not seen with beta-cyfluthrin, which contained virtually only isomers **II** and **IV**. As a consequence, the activity ratio between cyfluthrin and beta-cyfluthrin is 1·3 instead of the expected value of 2·4 (Table 4). This means that under field conditions, especially with resistant pests, the expected improvement of performance by using beta-cyfluthrin instead of cyfluthrin cannot always be achieved. As in the case of the resistant Bang I strain, the activity of cyfluthrin is due not only to the activity of its isomeric constituents, but also to the 'inactive' isomer(s). In the case of Bang I, isomer **III** apparently contributed to the efficiency of cyfluthrin through a synergistic effect on the activity of isomer(s) **II** and **IV**.

It may be speculated that the 'inactive' isomers interfered with biochemical detoxification reactions in a competitive fashion, i.e. they appeared to protect the active isomers from degradation. Similar speculations were made by Ernst and Dittrich[13] who suggested that the inactive isomers in cypermethrin may serve as alternative substrates for pyrethroid esterases in larvae of bollworm species.

4.4 *Trans* isomers and insecticide resistance

Table 3 shows that resistance factors of the *trans* isomers of cyfluthrin are lower than the corresponding factors of the *cis* isomers. This is consistent with the hypothesis that, at least in the insect strains tested here, the biologically active *trans* isomer was less susceptible to resistance than the biologically active *cis* isomer.

Similar findings were reported by Ahmad and McCaffery[14] for *Helicoverpa armigera* (Hübner) with cypermethrin. The *cis*-isomer remained more active than the *trans* isomer in the susceptible as well as in the resistant strain. However, the resistance factors were 39 for *cis*-cypermethrin and only 20 for *trans*-cypermethrin. The biochemical basis of this stereoselective resistance phenomenon was elucidated for *H. virescens* by McCaffery *et al.*[15] who found the metabolism of *cis*-cypermethrin to occur faster than that of *trans*-cypermethrin.

Since cyfluthrin and beta-cyfluthrin belong to the group of commercial insecticides which contain a high portion of *trans* isomers ('high *trans* pyrethroids'), their superior performance became evident as resistant strains of lepidopteran pests were investigated. This was also later found by other research groups such as Campanhola and Plapp[16] who compared the efficacy of pyrethroids on susceptible and resistant strains of tobacco budworm. If the efficacies were compared using a susceptible strain, deltamethrin performed better than cyfluthrin and the other major competitive pyrethroids. However, this pattern changed when a resistant strain originating from various cotton fields in the United States, and maintained under selection pressure of permethrin and cypermethrin, was used. Although cross-resistance between the pyrethroids was observed, the

performance of cyfluthrin was superior to that of other important commercial insecticides.

The high activity of the cyfluthrins on the major pest in Australian cotton, *H. armigera*, which was investigated by Forrester *et al.*,[17] may also be partially due to the high content of *trans* isomers in both products. Among the commercial insecticides investigated, cyfluthrin and beta-cyfluthrin performed best, especially in resistant larvae. With alpha-cypermethrin the resistance factor for the *cis* form was 9, slightly higher than the factor of 7·2 for the *trans* isomer.

Further support demonstrating the good performance of *trans* isomers against pyrethroid-resistant insect strains came from Ernst and Dittrich.[13] Using a leaf-dipping test, they investigated neonate larvae of new world bollworm species such as *H. virescens*, *Helicoverpa zea* (Boddie) and *H. armigera*. Two batches of cypermethrins were compared: a 'normal' one had a *cis*/*trans* isomer ratio of 40/60 while another, 'high *cis*', batch had a ratio of 80/20. Field strains from Nicaragua, Columbia and Guatemala showed resistance factors of 14, 45 and 53 for the 'normal' cypermethrin in contrast to 49, 90 and 134 with the 'high *cis*' cypermethrin. This indicated indirectly that, in this case, the *trans* isomers were also less prone to insect resistance.

ACKNOWLEDGEMENT

This work was performed in the former laboratory of 'Arthropod Resistance'. The excellent technical assistance of Annette Jacobs, Martina Weile and Michael Geppert, who performed the above experiments, is highly appreciated. We are grateful to Dr Meisner and his colleagues for rearing the insects, Mr Nauen for critically reviewing the paper and Dr Anderson for improving the style of the paper.

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